receptor X were transfected into CHO cells. The CHO transfectants were characterized by FACS and then scaled up for KinExA binding studies. KinExA has been used to measure binding affinity of Adnectin-A to the cell surface expressed receptor X to measure the effect of avidity of the multivalent adnectin binding to receptor clusters. As controls for the functional activity of the Adnectin-A and the affinity of the monovalent interaction, the same KinExa assay was used, substituting the soluble receptor X extracellular domain for the transfected CHO cells. The binding avidity measured by KinExA for CHO expressed receptor is 14 pM for both species of receptor X. However, the affinity of Adnectin-A for monovalent soluble Receptor X was quite different between the species suggesting that avidity due to receptor clustering equilizes the functional avidity at the cell surface.

Platform: Voltage-gated K Channels: Activation/ Inactivation Mechanisms

92-Plat

A-Type Kv4 Channel Closed-State Inactivation is Modulated by the Tetramerization Domain Interacting with Auxilary KChIP4a

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A-type Kv4 potassium channels undergo a conformational change towards a non-conductive state at negative membrane potentials, a dynamic process known as closed-state inactivation (CSI). CSI causes inhibition of channel activity without prerequisite of channel opening, thus providing a dynamic regulation of neuronal excitability, dendritic signal integration and synaptic plasticity. However, the structural determinants underlying Kv4 CSI remain largely unknown. We have recently demonstrated that auxiliary KChIP4a subunit contains an N-terminal Kv4 inhibitory domain (KID) that directly interacts with Kv4.3 channels to enhance CSI. In this study, we utilized the FRET two-hybrid mapping and BiFC-based screening combined with electrophysiology, and identified the intracellular tetramerization (T1) domain that functions to suppress CSI and serves as a receptor for the binding of KID. Disrupting Kv4.3 T1-T1 interaction by mutating C110A within the C3H1 motif of T1 domain facilitated CSI, and ablated the KID-mediated enhancement of CSI. Furthermore, replacing the characteristic C3H1 motif of Kv4.3 T1 domain with the T1 domain from Kv1.4 without the C3H1 motif or Kv2.1 with the C3H1 motif resulted in channels functioning with enhanced or suppressed CSI, respectively. Taken together, our findings reveal a novel role of the T1 domain in suppressing Kv4 CSI with the C3H1 motif functioning to stabilize the channel activation gate; and KChIP4a KID directly interacts with the T1 domain to relieve the stabilization, leading to facilitation of CSI and inhibition of channel function.

93-Plat

Two-in-One: Activation and Inactivation at the Intracellular Gate of a Kv Channel

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N-type and P/C-type inactivation are firmly established mechanisms of inactivation in voltage-gated K^+ (Kv channels). However, Kv4.x channel complexes, which undergo fast preferential closed-state inactivation (CSI; Fineberg et al., 2012, *JGP* 140.5:513-527), appear to use a distinct but unknown inactivation mechanism. Previously, we hypothesized that a weak interaction between the voltage sensing domain and the intracellular activation gate underlies CSI (Bähring & Covarrubias, 2011, J Physiol 589:461-79). Thus, CSI is essentially governed by the intracellular activation gate, which fails to open and adopts an inactivated conformation. To directly test this hypothesis, we investigated the heterologously expressed Kv4.1 ternary channel complex including accessory subunits KChIP1 and DPP6, and exploited the ''trap-door'' paradigm of the activation gate. The results show that Kv4.1 inactivation traps intracellularly applied quaternary ammonium blockers (bTBuA and TBuA) inside the channel's pore. The trapped blockers can only escape if the channels are opened again by subsequent depolarizations. By contrast, inactivation cannot trap TEA, whose binding kinetics is faster than that of channel gating. Moreover, under identical conditions, a Shaker Kv channel (ShB-T449K) known to exhibit fast P/C-type inactivation cannot trap bTBuA. These findings conclusively suggest that the intracellular activation gate of the Kv4.1 ternary channel complex plays a novel dual role, controlling both activation and inactivation. Supported in part by NIH grant R01 NS032337 (MC).

94-Plat

Development and Validation Studies of Universal Pharmacophore Models for hERG Channel Openers

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The intra-cavitary drug blockade of hERG channel, a common off-target for many drugs, have been extensively studied both experimentally and theoretically. Structurally diverse ligands inadvertent blockade of rapid component of delayed rectifying K^+ currents are potentially pro-arrhythmic and may lead to drug-induced long QT syndrome-LQTS. There are a number of natural strategies for rational drug design; one dubbed the ''passive'' approach avoids block of hERG1 whereas the ''proactive'' strategy designs treatments to activate the channel. While ''passive'' approach has been developed for decades, studies of structural mechanisms of hERG channel activation by small molecules are truly novel. Accordingly, design of the hERG openers or current activators may offer a momentum for modern anti-arrhythmia drug development. Significant number of small molecules with capacity for hERG activation was identified in mandatory hERG screens. To establish possible correlation between activators structure and reactivity, we attempted to construct a universal pharmacophore model for hERG channel openers using PHASE protocol. The biochemical data on 38 K^+ channel activators are used in training and test sets. These compounds span a wide range of structurally different chemotypes with ~10^5-fold variances in binding affinity, which is sufficient for statistically sound model. A developed five sites AAHHR (A, hydrogen-bond accepting, H, hydrophobic, R, aromatic) pharmacophore model has showed reasonable high statistical results compared to other constructed models and was selected for steric and electrostatic contour maps analysis. The predictive power of the model was also tested with 6 external test-set (as true unknowns) compounds. Pharmacophore model is also combined with previously developed receptor-based homology model of hERG K channel and novel activators are generated and screened. The developed ligand-based models may serve as a basis for the synthesis of novel potential therapeutic hERG activators.

95-Plat

N-Terminal Regulation of hERG1 K^+ Channel Deactivation

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Slow deactivation of hERG1 (Kv 11.1) potassium channels maintains I_{Kr} during final repolarization of the cardiac action potential and opposes asynchronous early depolarization. Inherited point mutations in hERG1 that accelerate deactivation of I_{Kr} cause long QT syndrome (LQTS), a disorder of ventricular repolarization that increases the risk of lethal cardiac arrhythmia.

The intracellular N-terminal domain of hERG1 is known to be essential for slow deactivation. Deletion of the entire (~350 residues) or just the initial 16 residues of the N-terminus accelerates deactivation 10-fold. The same effect is achieved by neutralization of the charged residues, Arg4 or Arg5. How many of the 4 N-termini are required to slow channel deactivation is unknown. hERG1, like other Kv channels, is a homotetramer. By repeatedly linking the C-terminal of one subunit to the N-terminal of the next subunit we constructed concatenated hERG1 tetramers. A variety of homomeric and heteromeric concatenated tetramers were characterized (i.e., WTn/R4A:R5A(4-n); where n = 1 to 4). The concatenated channel containing a single R4A/R5A subunit and 3 wild-type subunits deactivated as fast as the concatenated channel containing only R4A/R5A subunits. The LQTS-associated mutation R56Q, located in the N-terminal of hERG1 was also studied. Again, a concatenated tetramer containing a single mutant subunit deactivated as fast as channels with R56Q mutations in all four subunits. Our results show that all 4 N-termini are required to mediate slow deactivation in wild-type hERG1 channels.

96-Plat

Lipid Affinity to the Voltage-Gated Potassium Channel KvAP

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Voltage-gated potassium channels (KV) are formed by a central conducting pore surrounded by four voltage sensor domains. Functional studies have revealed that biophysical properties of lipid molecules in the channels environment can have strong effects on the activity of KV channels. Here, we investigated the influence of different lipids as well as their affinity to KvAP channels. We carried out electrophysiology measurements by fusing vesicles containing purified channels into planar lipid bilayers with varied lipid compositions. We found that KvAP properties are mainly determined by the lipid

composition in the vesicles and not the lipids in the bulk bilayer, suggesting that KvAP has a preferential affinity to the lipids it comes in contact with first. It also shows that there is very limited exchange of the annular lipids around the channels. Even with time and at higher temperature, lipids from the bilayer did not mix with those of the vesicles, suggesting a strong channel-lipid interaction or confinement of lipid molecules to a microdomain around the channel. The annular lipids in immediate proximity of the VSD determines the mid-activation point of KvAP. Additionally, a bulk effect from positively charged lipids forming the bilayer affected the slope of the conductance-voltage curves. Here, the energy barriers and thus the kinetic rates of pore opening and entering the inactivated state are affected. This effect could be neutralized by addition of counter charges to the bilayer. Our results suggest that the binding site proposed also for other Kv channels has a similar affinity for different phospho- and non-phospholipids whereas exchange with bulk lipids is very restricted. Apart from the structural information, this has also implications on the experimental design when working with membrane proteins reconstituted in planar lipid bilayer.

97-Plat
A Structural Driven Kinetic Cycle for KcsA Gating
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In an attempt to understand at the atomic level the structure-function correlations that define a working K^+ channel, it would be ideal to provide structural snapshots of a given channel as it transitions along its kinetic cycle. Here, we present a full atomic description for the kinetic cycle of KcsA, the archetypal pore domain of a K^+ channel. The simplest kinetic cycle that defines the function of KcsA involves its transition through at least 4 distinct kinetic states: closed-conductive (C/O) $^{\rightarrow}$ open-conductive (O/O) $^{\rightarrow}$ open-C-type inactivated (O/I) $^{\rightarrow}$ closed-C-type inactivated (O/I). We incorporated in a 4-state kinetic cycle (a) the high- and low-K^+ concentration structures of KcsA, believed to represent structural snapshots for KcsA's C/O and C/I states, respectively1; and (b) two new and unpublished structures of KcsA with the activation gate locked-open, while bearing a point mutation that either obliterates (E71A) or accelerates (Y82A) C-type inactivation. This allowed us to complete the simplest structure-driven kinetic cycle for KcsA. The high-resolution structures of KcsA's O/O and O/I states provide unparalleled structural information that lends a novel molecular explanation for C-type inactivation gating. A comprehensive functional and structural analysis will be presented.
Funding: L.G.C. NIH 1RO1GMo97159-01A1 and Welch Foundation BI-1757
1 Zhou, M., Morais-Cabral, J. H., Mann, S. & MacKinnon, R. Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. Nature 411, 657-661, doi:10.1038/3507950035079500 [pii] (2001).

98-Plat
State-Dependent Crosslinking in IKS Demonstrates a Closed-State Interaction between KCNE1 at F57 and KCNQ1 that Inhibits Channel Opening
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The slow delayed rectifier current (I_{Ks}) is a key repolarizing potassium current in the cardiac action potential. I_{Ks} is composed of KCNQ1 which forms the tetrameric voltage gated pore subunit and KCNE1, a single transmembrane domain accessory subunit, proposed to reside in the channel's exterior cleft. KCNE1 imposes a dramatic regulation on KCNQ1, significantly delaying opening compared to the unchaperoned channel. Here, we have investigated this interaction using the UV-crosslinking unnatural amino acid, *p*-benzoyl-L-phenyl alanine (*p*Bpa). *p*Bpa was genetically incorporated into KCNE1 at residue F57 in the transmembrane domain using the amber stop codon (TAG) suppression system. Successful incorporation into KCNE1-F57TAG was confirmed by a lack of functional I_{Ks} current in transfected cells not supplemented with *p*Bpa. Characterization of the *p*Bpa-incorporated channel complex revealed a right shifted $V_{0.5}$ of activation compared to wild-type (+27 mV vs. +14 mV). Channels were UV-irradiated in the closed state by applying a 300 ms light pulse at −90 mV followed by a 4s activation step (+60 mV). A diary plot of the peak current vs. UV-exposure with repeated exposure revealed a rapid decrease in available current compared to UV-treated wild-type channels. This indicates the permanent closure of channels by crosslinking. Application of UV at the end of a 4s activation step (+60 mV) produced an immediate downward deflection in current and resulted in a slower rate of channel crosslinking compared to the closed-state UV treatment. This suggests that KCNE1 can revisit a closed-state orientation during channel activation and is then trapped in the closed-state by the covalent crosslink. These findings provide new insight into the interactions that regulate the I_{Ks} channel complex.

99-Plat
Moving Gating Charges through the Gating Pore in a Kv Channel Voltage-Sensor
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S4-based Voltage-Sensor Domains (VSD) regulate ion channels and enzymes by transporting electrically-charged residues across a hydrophobic VSD constriction referred to as ''gating pore'' or ''hydrophobic plug''. How the gating pore controls the gating charge movement remains presently debated. Here, by characterizing over 100 gating pore mutations in the Shaker Kv channel VSD using gating current measurements, we uncovered non-ambiguous quantitative correlations between the voltage-dependence and kinetics of the VSD movement and the hydrophobicity or size of the side chains present at specific positions. Importantly, our results strongly suggest that a necessary small residue at position S240 (in S1) creates a ''steric gap'' that delineates an intracellular access pathway for transport of the gating charges. In addition, two large side chains at positions F290 (in S2) and F244 (in S1) contribute to stabilize the VSD in its activated conformation using a ''molecular clamp'' mechanism. This process is strengthened by a hydrophobic lateral chain at position I237 (in S1) acting as an intracellular ''hydrophobic wedge'' that impedes deactivation of the gating charges. This work sheds light on critical physicochemical principles underlying the transduction of electrical signals by voltage-sensor domains. Supported by NIH-GM030376.

Platform: Protein-Lipid Interactions I

100-Plat
Replica Exchange Umbrella Sampling Simulations Provide Insight into the Role of Docosahexaenoic Acid in Modulating the Stability of Transmembrane Proteins
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Polyunsaturated fatty acids are known to dramatically change the structural and dynamic properties of membrane bilayers, and to modulate the properties of membrane proteins, most notably the GPCR Rhodopsin that resides in rod outer cell membranes greatly enriched in docosahexaenoic acid (DHA). We previously showed that DHA exhibits unique lipid-protein interactions with Rhodopsin and hypothesized that increased DHA-Rhodopsin contacts come at the expense of helix-helix interactions in the protein. This model explains the experimentally observed lowering of Rhodopsin's unfolding temperature with increasing polyunsaturation and suggests a novel mechanism by which DHA lowers the energy of activation to the light adapted state by destabilizing the ground state. To further test this idea we have constructed MD simulations of model transmembrane helices in lipid bilayers composed of saturated dipalmitoylphosphatidylcholine (DPPC) and of polyunsaturated 1-palmitoyl-2-docosahexaenoylphosphatidylcholine (PDPC), lipids chosen based on their essentially identical hydrophobic thickness. For each lipid we have computed the potential of mean force as a function of helix-helix distance. To address the substantial sampling challenges posed by slow relaxation of peptide and lipid conformations we employed replica exchange umbrella sampling after generating independent lipid starting configurations for each window. Using thermodynamic integration, with mean forces averaged over greater than one microsecond of simulation, we found a significant difference for the dimerization energy of the two lipids. The helix-helix association energy is greatly diminished in the DHA containing membrane, consistent with our stated hypothesis. By decomposing the mean force into contributions from helix-helix and membrane-helix interactions we found that DHA disrupts attractive interactions between the helices. These results highlight the importance of the lipid bilayer, and in particular short-range direct lipid-protein interactions, in modulating the structure and function of membrane proteins.

101-Plat
Proton-Coupled Water and Hydrogen-Bond Dynamics in Channelrhodopsin
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Channelrhodopsin is a light-gated cation channel whose reaction cycle involves proton-transfer reactions. Understanding how channelrhodopsin works is